D ck t No. 68518-A/JPW/GJG/JBC

Application
for
United States Letters Patent

To all whom it may concern:

Be it known that

Sharon Cohen-Vered, Esmira Naftali, Vera Weinstein, Adrian Gilbert and Ety Klinger

have invented certain new and useful improvements in

PARENTERAL FORMULATIONS OF A PEPTIDE FOR THE TREATMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS

of which the following is a full, clear and exact description.

PARENTERAL FORMULATIONS OF A PEPTIDE FOR THE TREATMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS

This application claims the benefit of U.S. Provisional Application No. 60/439,950, filed January 14, 2003, the entire contents of which are hereby incorporated by reference.

Throughout this application, various publications are referenced by full citations. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

Background of the Invention

Systemic lupus erythematosus (SLE), or lupus, is a debilitating autoimmune disease characterized by the presence of an array of autoantibodies, including antibodies to dsDNA, to nuclear antigens and to ribonucleoproteins. SLE affects approximately 1 in 2000 individuals (U.S. 1 in 700 women). The disease primarily affects young women, with a female-to male ratio of approximately 9:1.

Systemic lupus can affect almost any organ or system of the body. Systemic lupus may include periods in which few, if any, symptoms are evident ("remission") and other times when the disease becomes more active ("flare"). Most often when people mention "lupus," they are referring to the systemic form of the disease.

Corticosteroids are the mainstay in treating systemic autoimmune disorders. Life threatening, severely disabling manifestations of SLE are treated with high doses of

glucocorticoids (1-2 mg/kg/day). Undesirable effects of chronic glucocorticoids include an array of prominent adverse effects such as cushingoid habitus, central obesity, hypertension, infection, capillary fragility, hirsutism, accelerated osteoporosis, cataracts, diabetes mellitus, myopathy and psychosis. In addition to corticosteroid toxicity, patient compliance to a dosage regimen also poses a serious problem.

Cytotoxic agents are also used for controlling active disease, reducing the rate of disease flares, and reducing steroid requirements. Undesirable side effects of the latter include bone marrow depression, increased infections with opportunistic organisms, irreversible ovarian failure, alopecia and increased risk of malignancy.

SLE is an inflammatory disease for which to date there is no definitive treatment or cure. The disease results in acute and chronic complications. The only treatments available are palliative, aimed at relieving acute symptoms and preventing chronic complications, often with profound side effects. There is therefore an unmet need in this field, and both physicians and patients would welcome new treatments which could potentially eliminate or reduce the unwanted manifestations of the disease.

Peptides based on the complementarity-determining region of the human monoclonal anti-DNA 16/6Id antibody capable of immunomodulating SLE associated responses have been disclosed in PCT International Publication No. WO 02/067848 A2, the entire contents of which are hereby incorporated by reference. In particular, region CDR1 was found to inhibit the proliferative response of peripheral blood lymphocytes (PBL) of SLE patients to the human anti-DNA 16/6Id mAB, and to

ameliorate disease manifestations of mice afflicted with spontaneous or experimental SLE.

Human CDR1, Compound 1, shown in Figure 1, is a synthetic peptide of 19 amino acids based on the complementarity-determining region 1 (CDR1) of the human anti-dsDNA mAb denoted 16/6 Id (Waisman, A., et al. "Modulation of murine systemic lupus erythematosus with peptides based on complementarity determining regions of pathogenic anti-DNA monoclonal antibodies." *Proc. Natl. Acad. Sci. U.S.A.* (1997), 94(4): 4620-4625).

In experimental SLE models - Balb/c mice and SLE-prone mice, i.e. (NZBxNZW)F1 mice - treatment with either mCDR based-peptides or Compound 1 significantly reduced the SLE related findings, notably immune complex deposits (ICD) in the kidney, proteinuria and leukopenia. The treatment had no effect on the 16/6 Id specific antibody response (Waisman, A., et al. "Modulation of murine systemic lupus erythematosus with peptides based on complementarity determining regions of pathogenic anti -DNA monoclonal antibodies." *Proc. Natl. Acad. Sci. U.S.A.* (1997), 94(4): 620; Eilat, E., et al., "Prevention of systemic lupus erythematosus-like disease in (NZBxNZW)F1 mice by treating with CDR1- and CDR3- based peptides of pathogenic autoantibody" *J. Clin. Immunol.* (2000), 20: 268; Eilat, E., et al., "The mechanism by which a peptide based on complementarity determining region-1 of pathogenic anti-DNA antibody ameliorates experimental SLE" (2001), *Proc.Natl.Acad. Sci. U.S.A.* 98: 1148).

These peptides, like many peptides, are not very soluble. Therefore, formulations that improve the solubility of the peptides are desired.

Summary of Invention

The subject invention provides a pharmaceutical composition comprising

an aqueous carrier;

from 0.1 mg/ml to 20 mg/ml of the composition of a pharmaceutically acceptable salt of a peptide having the structural formula

NH_2-Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Glu Glu Trp Ile Gly-COOH (SEQ ID NO:1); and

a substituted β-cyclodextrin in an amount effective to dissolve the peptide in the aqueous carrier,

wherein the composition has a pH between 4 and 9.

The subject invention also provides a pharmaceutical composition comprising

an aqueous carrier;

from 0.1 mg/ml to 20 mg/ml of the composition of an acetate salt of a peptide having the structural formula

NH_2-Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Glu Glu Trp Ile Gly-COOH (SEQ ID NO:1);

and

from 70 mg/ml to 170 mg/ml of the composition of hepta-(sulfobutyl ether)-β-cyclodextrin,

wherein the peptide and the hepta-(sulfobutyl ether)-β-cyclodextrin are dissolved in the aqueous carrier; and

wherein the solution has a pH between 6.5 and 8.5.

The subject invention also provides a method of alleviating symptoms of systemic lupus erythematosus (SLE) in a human subject comprising administering to the human subject any of the above pharmaceutical compositions in an amount effective to alleviate the symptoms of SLE in the human subject.

The subject invention also provides a process for manufacturing the above pharmaceutical composition comprising the steps of:

a) preparing a solution of a substituted β-cyclodextrin in an aqueous carrier at a predetermined concentration;
b) adding predetermined amount of a pharmaceutically acceptable salt of the peptide NH_2-Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Glu Glu Trp Ile Gly-COOH (SEQ ID NO:1) to the solution of step a);
c) adjusting the pH of the solution of step b) until the peptide dissolves in the solution; and
d) if necessary, adjusting the pH of the solution of step c) to a pH of 4-9, thereby manufacturing the pharmaceutical composition.

The subject invention also provides a process of lyophilizing the above pharmaceutical composition, comprising the steps of:
a) lowering the temperature of the pharmaceutical composition to -40°C;
b) holding the temperature at -40°C for a predetermined time;
c) raising the temperature of the solution to 20°C;
d) holding the temperature at 20°C for a predetermined time; and
e) reducing the pressure to 10μbar, thereby lyophilizing the pharmaceutical composition.

The subject invention also provides a process of lyophilizing the above pharmaceutical composition, comprising the steps of:
a) lowering the temperature of the pharmaceutical composition to -45°C;
b) holding the temperature at -45°C for a predetermined time;
c) raising the temperature of the solution to -20°C;

d) raising the temperature of the solution to 25°C; and

e) holding the temperature at 25°C for a predetermined time, thereby lyophilizing the pharmaceutical composition.

Brief Description of Figures

Figure 1. Human CDR1 (Compound 1) as acetate salt - showing the molecular and structural formulas of hCDR1, the amino acid sequence, and physical parameters

Figure 2. IL-2 Secretion from cells taken from mice treated with Compound 1 and Captisol® solution after the cells were subsequently activated with a solution of Compound 1 in PBS.

-■- Compound 1 (RS) 50µg/mouse

-▲- Compound 1 (RS) 200µg/mouse

-□- DP 50µg/mouse

-Δ- DP 200µg/mouse

-●- 12% Captisol® ampulized

Figure 3. IFN-γ Secretion from cells taken from mice treated with Compound 1 solution after the cells were subsequently activated with a solution of compound 1 in EM-1 (2.5×10^6 cells/well).

-♦- Placebo

-●- Compound 1 50 µg/mouse (treatment dose)

-Δ- Compound 1 100 µg/mouse (treatment dose)

-X- Compound 1 200 µg/mouse (treatment dose)

Figure 4. IFN-γ Secretion from cells taken from mice treated with Compound 1 solution after the cells were subsequently activated with a solution of compound 1 in EM-1 (5×10^6 cells/well).

-♦- Placebo

-□- Compound 1 25 µg/mouse

-Δ- Compound 1 50 µg/mouse

-X- Compound 1 100 µg/mouse

-*- Compound 1 200 µg/mouse

Figure 5. Anti-dsDNA antibodies in (NZBxNZW)F1 mice after 10 injections with Compound 1 in Captisol® [OD=Optical Density; Compound 1 (C)= Compound 1 dissolved in Captisol®]

-□- Placebo

-◊- Compound 1 50 μg/mouse

-o- Compound 1 25 μg/mouse

Figure 6. Kidney sections from (NZBxNZW)F1 mice showing intensity of Immune Complex Deposits. The top row sections are from a Captisol®-treated mouse, the mid-row sections are from a mouse treated with 50 μg/mouse Compound 1 and the bottom row sections are from a mouse treated with 25 μg/mouse Compound 1. *Magnification*: Left: x100, Right: x400. FITC immunohistology.

Detailed description

The subject invention provides a pharmaceutical composition comprising

an aqueous carrier;

from 0.1 mg/ml to 20 mg/ml of the composition of a pharmaceutically acceptable salt of a peptide having the structural formula

NH_2-Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Glu Glu Trp Ile Gly-COOH (SEQ ID NO:1); and

a substituted β-cyclodextrin in an amount effective to dissolve the peptide in the aqueous carrier,

wherein the composition has a pH between 4 and 9.

In one embodiment, the concentration of the acetate salt of the peptide is at least 0.5 mg/ml.

In one embodiment, the concentration of the salt of the peptide is from 0.5 mg/ml to 10 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 0.5 mg/ml to 2.5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 2.5 mg/ml to 5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 5 mg/ml to 7 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 7 mg/ml to 8.5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 8.5 mg/ml to 10 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 9 mg/ml to 10 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 10 mg/ml to 15 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 15 mg/ml to 20 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 1.0 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 2.5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 10 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 15 mg/ml.

In another embodiment, the concentration of the salt is from 0.1 mg/ml to 0.5 mg/ml.

In another embodiment, the concentration of the salt is from 0.1 mg/ml to 0.2 mg/ml.

In another embodiment, the concentration of the salt is from 0.2 mg/ml to 0.3 mg/ml.

In another embodiment, the concentration of the salt is from 0.3 mg/ml to 0.4 mg/ml.

In another embodiment, the concentration of the salt is from 0.4 mg/ml to 0.5 mg/ml.

In a further embodiment, the composition has a pH between 6.5 and 8.5.

In a further embodiment, the composition has a pH between 7.5 and 8.5.

In a further embodiment, the composition has a pH between 4 and 5.

In a further embodiment, the composition has a pH between 5 and 6.

In a further embodiment, the composition has a pH between 6 and 7.

In a further embodiment, the composition has a pH between 7 and 8.

In a further embodiment, the composition has a pH between 8 and 9.

In another embodiment, the pharmaceutically acceptable salt is an acetate salt.

In another embodiment, the substituted β-cyclodextrin is a hydroxypropyl, a sulfobutyl ether, or a sulfopropyl ether substituted β-cyclodextrin.

In a further embodiment, the substituted β-cyclodextrin is a

sulfobutyl ether substituted β-cyclodextrin.

In a further embodiment, the pharmaceutically acceptable salt is an acetate salt, and the substituted β-cyclodextrin is hepta-(sulfobutyl ether)-β-cyclodextrin.

In another embodiment, the composition further comprises a pharmaceutically acceptable buffer in an amount and of a type suitable to make the pH of the pharmaceutical composition in the range of 4-9. The buffer may be acetate buffer, citrate buffer, or sodium carbonate.

The subject invention also provides a pharmaceutical composition comprising

an aqueous carrier;

from 0.1 mg/ml to 20 mg/ml of the composition of an acetate salt of a peptide having the structural formula

NH_2-Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Glu Glu Trp Ile Gly-COOH (SEQ ID NO:1);

and

from 70 mg/ml to 170 mg/ml of the composition of hepta-(sulfobutyl ether)-β-cyclodextrin,

wherein the peptide and the hepta-(sulfobutyl ether)-β-cyclodextrin are dissolved in the aqueous carrier; and

wherein the composition has a pH between 6.5 and 8.5.

In one embodiment, the concentration of the acetate salt of the peptide is at least 0.5 mg/ml.

In one embodiment, the concentration of the acetate salt of the peptide is from 0.5 mg/ml to 10 mg/ml.

In a further embodiment, the concentration of the acetate salt of the peptide is from 0.5 mg/ml to 2.5 mg/ml.

In another embodiment, the concentration of the salt is from 0.1 mg/ml to 0.5 mg/ml.

In another embodiment, the concentration of the salt is from 0.1 mg/ml to 0.2 mg/ml.

In another embodiment, the concentration of the salt is from 0.2 mg/ml to 0.3 mg/ml.

In another embodiment, the concentration of the salt is from 0.3 mg/ml to 0.4 mg/ml.

In another embodiment, the concentration of the salt is from 0.4 mg/ml to 0.5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 5 mg/ml to 7 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 7 mg/ml to 8.5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 8.5 mg/ml to 10 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 9 mg/ml to 10 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 10 mg/ml to 15 mg/ml.

In another embodiment, the concentration of the salt of the peptide is from 15 mg/ml to 20 mg/ml.

In a further embodiment, the concentration of acetate salt of the peptide is 1.0 mg/ml.

In a further embodiment, the concentration of acetate salt of the peptide is 2.5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 5 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 10 mg/ml.

In another embodiment, the concentration of the salt of the peptide is 15 mg/ml.

In another embodiment, the concentration of hepta-(sulfobutyl ether)-β-cyclodextrin is 120 mg/ml and the pH of the composition is between 7.5 and 8.5.

The subject invention also provides a method of alleviating symptoms of systemic lupus erythematosus (SLE) in a human subject comprising administering to the human subject any of the above pharmaceutical compositions in an amount effective to alleviate the symptoms of SLE in the human subject.

The subject invention also provides the above pharmaceutical compositions for use in treating SLE in a human subject.

The subject invention also provides a process for manufacturing any of the above pharmaceutical compositions comprising the steps of:

a) preparing a solution of a substituted β-cyclodextrin in an aqueous carrier at a predetermined concentration;

b) adding predetermined amount of a pharmaceutically acceptable salt of the peptide NH_2-Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Glu Glu Trp Ile Gly-COOH (SEQ ID NO:1) to the solution of step a);

c) adjusting the pH of the solution of step b) until the peptide dissolves in the solution; and

d) if necessary, adjusting the pH of the solution of step c) to a pH of 4-9, thereby manufacturing the pharmaceutical composition.

In one embodiment of the process, the resulting final concentration of the substituted β-cyclodextrin in the pharmaceutical composition is from 70 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 80 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 90 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 100 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 110 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 120 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 130 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 140 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 150 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition of from 160 mg/ml to 170 mg/ml.

In one embodiment of the process, the predetermined concentration of the substituted β-cyclodextrin is such which results in a final concentration of substituted β-cyclodextrin in the pharmaceutical composition is 120 mg/ml.

In another embodiment, the predetermined amount of peptide is such which results in a final concentration of peptide in the pharmaceutical composition is at least 0.1 mg/ml.

In another embodiment, the predetermined amount of peptide is such which results in a final concentration of peptide in the pharmaceutical composition is at least 0.5 mg/ml.

In another embodiment, the predetermined amount of peptide is such which results in a final concentration of peptide in the pharmaceutical composition is 2.5 mg/ml, 2.0mg/ml, 1.0mg/ml, 0.5 mg/ml or 0.1 mg/ml.

In another embodiment, the predetermined amount of peptide is such which results in a final concentration of peptide in the pharmaceutical composition is 5 mg/ml, 10 mg/ml or 15 mg/ml.

In another embodiment of the process, step b) further comprises mixing the solution for 1 hour.

In another embodiment, in step c) the pH is adjusted using HCl or NaOH 1.0N.

In another embodiment, the process further comprises filtering the solution of step d) through a cellulose acetate filter.

In another embodiment of the above process,

the predetermined concentration of the substituted β-cyclodextrin is such which results in a final

concentration of substituted β-cyclodextrin in the pharmaceutical composition is 120 mg/ml;

the predetermined amount of peptide is such which results in a final concentration of peptide in the pharmaceutical composition is 2.5 mg/ml, 2.0mg/ml, 1.0mg/ml, 0.5 mg/ml or 0.1 mg/ml;

step b) further comprises mixing the solution for 1 hour; and

in step c) the pH is adjusted using HCl or NaOH 1.0N,

and the process further comprises filtering the solution of step d) through a cellulose acetate filter.

The subject invention also provides a composition prepared by the above process.

The subject invention also provides a process of lyophilizing the above pharmaceutical composition, comprising the steps of:

a) lowering the temperature of the pharmaceutical composition to -40°C;
b) holding the temperature at -40°C for a predetermined time;
c) raising the temperature of the solution to 20°C;
d) holding the temperature at 20°C for a predetermined time; and
e) holding the temperature at 25°C for a predetermined time, thereby lyophilizing the pharmaceutical composition.

In one embodiment of the process, step a) is performed within 2 hours.

In another embodiment, step b) is performed within 3 hours.

In a further embodiment, step c) is performed over 13 hours.

In a further embodiment, step c) is performed at a pressure of 110μbar.

In a further embodiment, step d) is performed over 13 hours.

In a further embodiment, step d) is performed at a pressure of 110μbar.

In a further embodiment, in step e) the pressure is reduced to 10μbar.

In a further embodiment, step e) is performed over 5 hours.

In another embodiment of the process,

step a) is performed within 2 hours;

step b) is performed within 3 hours;

step c) is performed over 13 hours and at a pressure of 110μbar;

step d) is performed over 13 hours and at a pressure of 110μbar; and

step e) is performed over 5 hours and the pressure is reduced to 10μbar.

The subject invention also provides a lyophilized pharmaceutical composition prepared by the above process.

The subject invention also provides a process of lyophilizing the above pharmaceutical composition, comprising the steps of:

a) lowering the temperature of the pharmaceutical composition to -45°C;
b) holding the temperature at -45°C for a predetermined time;
c) raising the temperature of the solution to -20°C;
d) raising the temperature of the solution to 25°C; and

e) holding the temperature at 25°C for a predetermined time, thereby lyophilizing the pharmaceutical composition.

In one embodiment, step a) is performed within 6 hours.

In another embodiment, step b) is performed within 3 hours.

In another embodiment, step c) is performed over 19 hours.

In another embodiment, step c) is performed at a pressure of 150μbar.

In another embodiment, step d) is performed over 13 hours.

In another embodiment, step d) is performed at a pressure of 150μbar.

In another embodiment, step e) is performed over 8 hours.

In another embodiment, step e) is performed at a pressure of 150μbar.

In another embodiment of the process,

step a) is performed within 6 hours;

step b) is performed within 3 hours;

step c) is performed over 19 hours and at a pressure of 150μbar;

step d) is performed over 13 hours and at a pressure of 150μbar; and

step e) is performed over 8 hours and at a pressure of 150μbar.

The subject invention also provides a lyophilized pharmaceutical composition prepared by any of the above processes.

In one embodiment of the above lyophilized pharmaceutical composition, the water content of the composition is less than 5%.

In another embodiment, the water content of the composition is less than 4.0%.

In another embodiment, the water content of the composition is less then 3.5%.

The subject invention also provides a lyophilized pharmaceutical composition comprising

a pharmaceutically acceptable salt of a peptide having the structural formula

NH_2-Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Glu Glu Trp Ile Gly-COOH (SEQ ID NO:1); and

a substituted β-cyclodextrin.

The subject invention also provides a packaged pharmaceutical composition comprised of:

a packaging material; and

a predetermined amount of the above lyophilized pharmaceutical composition.

The preparations of the present invention may be given parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered by injection, inhalation, ointment, suppository, etc. administration by injection, infusion or

inhalation; topical by lotion or ointment; and rectal by suppositories.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systematically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

Details of general formulation procedures and information on additional excipients may be found in Remington: The Science and Practice of Pharmacy, 20th Edition.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Example 1: Formulation Development for Compound 1

The human hCDR1 peptide (Compound 1) is described in PCT International Publication No WO 02/067848, published September 6, 2002, and can be prepared by methods well known in the art, (see, for example, Peptides: Synthesis, Structure and Applications, ed. by B. Gutte, Academic Press, 1995; Peptide Synthesis Protocols, ed. By M. Pennington and B. Dunn, Humana Press, 1994; Schnolzer, M. et al., "In situ neutralization in Boc-chemistry solid phase synthesis. Rapid, High yield assembly of difficult sequences." *Int. J. Pept. Protein Res.* (1992) 40: 180-193).

Compound 1 is a synthetic polypeptide composed of 19 amino acids. It is provided as an acetate salt. The aqueous solubility of the peptide has been determined to be less than 0.5 mg/ml. Figure 1 shows compound 1 as an acetate salt.

In order to develop a formulation with peptide concentration exceeding 2 mg/ml, preferably up to 10 mg/ml, experiments with several solubility enhancers were performed. The preliminary experiments indicated that a concentration of 2 mg/ml cannot be easily attained. In order to develop a formulation for sub-cutaneous injection, it is also desirable that the pH be in the range of 4 to 9 and that the solution be iso-osmotic.

Based on an extensive literature survey, a few principal approaches were adopted in order to produce a formulation with maximal solubility. The factors considered were:

- pH adjustment and buffers
- Solvents
- Co-solvents

• Solubilizing agents

Methods

Compound 1 was dissolved in the chosen solubility enhancer solution either separately or in combination with other excipients and the solutions were stirred for at least an hour. The pH was adjusted if needed. The solutions were visually examined to estimate the solubility and sent for analytical assay determination. For a few chosen formulations, biological activity was also tested.

Results

Table 1 presents the type of solubility enhancers used for the formulation development. Tables 2 and 3 summarize the experiments that were performed with the various solubility enhancers. Table 2 summarizes the initial screening performed with peptide concentrations in the range of 5 to 10 mg/ml. The experimental work that was performed with higher peptide concentration was then repeated with the lower doses (see table 3)

Initial tests indicated that Compound 1 was more soluble at the limits of the desired pH levels, both acidic and basic, but was less stable at the basic pH range. Thus, several buffers and pH adjustment agents were tested, including acetate buffer, citrate buffer and sodium carbonate. None of the initially tested buffers achieved the desired peptide solubility level. Only above pH 9.2 and below pH 3.0 were solubility levels of 2 mg/ml observed. Nevertheless, at the initial stage, formulations with acetate buffer and citrate buffer (with Mannitol as a tonicity agent) were selected for initial toxicology studies. These formulations were tested for biological activity and proven active.

Non aqueous solvents (see table 1) such as Ethanol, Glycerin, Propylene glycol, Chremophore and their combinations were tested but did not increase the solubility of Compound 1. A solution of 30% DMA (dimethyl-acetamide) yielded solubility in the desired ranges (5 to 9 mg/ml), but was not suitable for a pharmaceutical formulation due to its toxicity profile. Improved solubility was also observed using 30% (w/w) PEG 400 (5 to 9 mg/ml). This latter formulation was chosen for the toxicology studies, but it has proved to be both inactive in the biological assay, and may have been the cause of some adverse effects in a mouse toxicity study. Thus, it was decided not to further pursue this formulation. In view of the preliminary experiments non-aqueous solvents were not used in the subject formulations.

Several amino acids (see table 1) including L-Arginine, L-Glutamic acid, L-Glycine and L-Lysine were tested to improve the protein solubility. The solubility of the peptide in L-Arginine was at the desired level but the resulting pH was above 9. An attempt to decrease the pH or use an Arginine HCl salt resulted in precipitation of the peptide. Human Serum Albumin was also tested and improved the solubility of the peptide at low peptide concentrations (1 mg/ml) (see table 3). However, due to its potential immunogenicity and the low peptide solubility, it was not utilized in further experiments.

Bulking agents (see table 1) including Mannitol, Sorbitol and Dextran were tested alone and in combination with other excipients, but did not improve the solubility of the peptide in solution.

Co-solvents (see table 1), including Polysorbate 20 and Polysorbate 80 were tested alone and in combination with other excipients. While lower concentrations of Polysorbates (up to 6%) did not improve the solubility of the peptide, higher concentrations (up to 10% - see table 2) improved the solubility of the peptide up to 2 mg/ml. However, such high concentrations of Polysorbates were deemed unsuitable for pharmaceutical formulations.

Two types of cyclodextrins, both approved for use in marketed parenteral products, were also tested: Hydroxypropyl-β-cyclodextrin and Sulfobutylether-β-cyclodextrin (Captisol®). Both markedly increased the solubility of the peptide (concentrations in the levels of 10 mg/ml for Hydroxypropyl-β-cyclodextrin and 2.5 for Captisol®). The biological activity of the two cyclodextrin formulations was tested and was found to be equal to the activity of the peptide alone.

CAPTISOL® is a commercially available polyanionic β-cyclodextrin derivative with a sodium sulfonate salt separated from the hydrophobic cavity by a butyl ether spacer group, or sulfobutylether (SBE). CAPTISOL® is the trade name for CyDex Inc.'s hepta-substituted sulfobutylether β-cyclodextrin (SBE7-β-CD) preparation (www.captisol.com). The structure of CAPTISOL® allows drug molecules to fit in the hydrophobic cavity, thereby isolating the drug molecule from the aqueous solvent. Because the outer surface of CAPTISOL® is hydrophilic, the solubility of the complexed drug molecule is thereby enhanced. The use of cyclodextrins to enhance the solubility of drug molecules is disclosed in U.S. Patent Nos. 5,134,127 and 5,376,645, the entire contents of which are hereby incorporated by reference.

According to the literature of CyDex Inc., CAPTISOL® is safe when administered parenterally and does not exhibit the nephrotoxicity associated with beta-cyclodextrin. Relative to beta-cyclodextrin, CAPTISOL® provides comparable or higher complexation characteristics and superior water solubility in excess of 90 grams/100ml - a 50-fold improvement.

Conclusions

Several solubility enhancers were found to match the desired solubility range: DMA, PEG-400, dimethyl-acetamide, polyethylene glycol, polyoxylated castor oil, N-methyl-2-pyrrolidinone, 1-ethenyl-2-pyrrolidinone, Polysorbate 20, Polysorbate 80, Hydroxypropyl-β-cyclodextrin and Sulfobutylether-β-cycldextrin (Captisol®). Of these solubility enhancers both cyclodextrins have proven to be superior with respect to solubility, biological activity and stability. Thus, it was decided to select Captisol® as the solubility enhancer for use in Example 5 formulations and to further study both cyclodextrin formulations. The final formulation for the Example 5 clinical studies consists of: 120 mg/ml of Captisol® in water with the desired amount of peptide (0.5, 1.0 or 2.5 mg/ml), and HCl and NaOH for pH adjustment.

Table 1: Solubility enhancers used for Compound 1 formulation development

Solubility enhancer classification	Solubility Enhancers
Solvents	Cremophor EL, CMC, Ethanol, DMA, Gycerin, Propylene Glycol, PEG 400, Monotioglycerol
Co-solvents	Polysorbate 20, Ploysorbate 80
Solubilizing agents	Argenine, HSA, Glycine, Creatinine, Glutamic acid, Lysine (acetate salt and free base), Captisol®, Hydroxypropyl-β-cyclodextrin,
Bulking agents	Mannitol, Sorbitol, Dextrose, Lactose Dextran
pH Adjustment Agents	Citrate buffer, Acetate buffer, Sodium Carbonate

Tabl 2. List of Cosolvents and Stabilizers evaluated in Compound 1 Peptide Formulations.

Solubility Enhancer *	*% Used*	*% of Standard amount from the literature*	*Amount of peptide added (mg/ml)*	*Assay,%*	*pH of formulation*	*Remarks*
Albumin (HSA), Dextrose	1.5 1.5	0.4-5.0	5		6.0 Adjust. to 4.1	Insoluble
Albumin (HSA), Polysorbate 80, Glycine	1.0 0.6 2.0	0.4-5.0 0.8-4.0 0.2-2.1	5	-	5.8 Adjust. to 4.1	Insoluble
Arginine	1.5	0.8-1.6	15	93	9.8	Clear solution
Arginine HCl	2.0	0.8-1.6	5	-	3.5	Insoluble
Arginine Lactose	1.5 1.5	0.8-1.6	15	93	9.8	When the pH was lowered below 8.5 the peptide precipitated and the solution turned into gel
Captisol®	10.0 20.0	Up to 30.0	10	86 89	4.9 Adjust. to 4.4	Turbid solution
CMC (carboxy methyl cellulose) in acetate	0.2 0.05M		5	90	5.0	For toxicology studies
Creatanine	0.8	Up to 0.6	5	-	6.1 Adjust. to 4.1	Insoluble
Cremophor EL Ethanol Dimethylacetamide	15.0 10.0 6.0	– 10.0 0.6-32.9 0.012-6.0	5	-	4.0	Very turbid
Dextran	4.0 to 15.0	3.0-30.0	5	-	3.9	Insoluble
Dimethylacetamide (DMA)	6.0-20.0	0.012-6.0	5	-	4.6	Insoluble

Solubility Enhancer *	*% Used*	*% of Standard amount*	*Amount of peptide added (mg/ml)*	*Assay,%*	*pH of formulation*	*Remarks*
Dimethylacetamide (DMA)	25.0	0.012-6.0	5	87	5.1	Clear solution
Dimethylacetamide (DMA)	30.0	0.012-6.0	10	93	5.1	Clear solution
Ethanol	10.0	0.6-32.9	5	-	-	Insoluble
Glutamic acid	2.0		5	-	3.7	When the pH was increased above 4 the peptide precipitated and the solution turned into gel
Glycerin	1.5	1.6-32.5	5	37	4.5	Insoluble
Glycerin	30.0	1.6-32.5	5	-	3.7	Insoluble
Glycerin, Polysorbate 80	10.0 0.6	1.6-32.5 0.8-4	5	12	6.5 Adjust. to 4.5	Insoluble
Glycine	0.4	0.2-2.1	5	-	4.6	Insoluble
Hydroxypropyl β-cyclodextrin	20.0	Up to 30.0	10	99	4.6	Clear solution
Lysine Acetate Salt	2.0		5	-	3.8	Insoluble
Lysine Free base	2.0		5	-	9.2	When the pH was lowered below 8 the peptide precipitated and the solution turned into gel
Mannitol in Citrate buffer	4.0 0.035M	2.0-10.0	5	38	3.4	For toxicology studies
Mannitol in acetate buffer	4.0 0.05M	2.0-10.0	5	32	4.3	For toxicology studies
Mannitol, Glycine	20.0 0.4	2.0-10.0 0.2-2.1	5	14	6.4 Adjust. to 4.5	Insoluble
Mannitol, Polysorbate 20	20.0 0.6	2.0-10.0 -	5	22	6.5 Adjust. to 4.5	Insoluble
Monothioglycerol	1.0	0.1-10.0	5	-	4.5	Turbid solution
PEG 400	30.0	Up to 30.0	5	88	4.2	Slightly opalescent

Solubility Enhancer *	*% Used*	*% of Standard amount from the literature*	*Amount of peptide added (mg/ml)*	*Assay,%*	*pH of formulation*	*Remarks*
PEG 400	30.0	Up to 30.0	10	89	4.2	Turbid solution
PEG 400 with DMA	30.0 6.0	Up to 30.0 0.012-0.6	5	94	4.2	Clear solution
PEG 400	10.0	6.0-18.0	5	58	4.2	Insoluble
PEG 400 DMA	10.0 10.0	Up to 30.0 0.012-0.6	5	-	4.3	Insoluble
PEG 400 Propylene glycol PG	10.0 10.0	Up to 30.0 10.0	5	-	4.1	Insoluble
PEG 400 Propylene glycol	18.0 50.0	Up to 30.0 10.0	5	100	4.2	Clear solution
Polysorbate 80	1.6	0.8-4.0	5	24	7.2 Adjust. to 4.5	Insoluble
Polysorbate 80	6.0	0.8-4.0	5	-	3.9	Insoluble
Polysorbate 80 Creatanine	6.0 0.6	0.8-4.0 up to 0.6	5	-	4.0	Insoluble
Propylene glycol PG DMA	10.0 10.0	10.0 0.012-0.6	5	-	4.2	Insoluble
Propylene glycol PG	10.0, 30.0	10.0	5	-	4.2	Insoluble
Sodium Carbonate	1.5		5	-	11.4	When the pH was lowered below 8.5 the peptide precipitated and the solution turned into gel
Sorbitol	5.0	10.0-25.0	5	-	6.9 Adjust. to 4.5	Turbid solution

Table 3: Compound 1 formulations at low peptide concentrations

Solubility Enhancer *	*% Used*	*% of Standard amount from the literature*	*Amount of peptide added (mg/ml)*	*Assay,%*	*pH of formulation*	*Remarks*
Albumin (HSA),	5.0	0.4-5.0	1.0	90	6.9	Clear solution
			2.5	-	Adjust. to 4.5	Turbid solution
Arginine	1.5	0.8-1.6	1.0	24	10.6 Adjust. to 8.5	When the pH was lowered below 8.5 the peptide precipitated and the solution turned into gel
Captisol®	12.0	Up to 30.0	1.0	106	5.3	Clear solution
			2.5	100	6.5 to 8.5	
Dextran	20.0	3.0-30.0	1.0	69	4.8 Adjust. to 4.0	Turbid solution
Glycerin	30.0	1.6-32.5	1.0	-	4.8 Adjust. to 4.0	Turbid solution
Mannitol	4.0	0.8-4.0	1.0	64	4.7 Adjust. to 4.0	Turbid solution
Polysorbate 20	10.0	-	1.0	95	5.8	Clear solution
			2.5	88	Adjust. to 4.8	Clear with small amount of precipitation
Polysorbate 20 Mannitol	10.0 2.0	- 2.0-10.0	2.5	115	5.1 Adjust. to 4.3	Clear solution
Polysorbate 80	4.0	0.8-4.0	1.0	91	5.5	Clear solution
	6.0-10.0		2.5	89	Adjust. to 5.0	Slightly turbid solution
Polysorbate 80 Mannitol	4.0 2.0	0.8-4.0 2.0-10.0	2.5	88	5.1 Adjust. to 4.4	Slightly turbid solution
Propylene glycol PG	10.0	10.0	1.0	78	5.0 Adjust. to 4.4	Turbid solution
Sorbitol	20.0	10.0-25	1.0	52	4.5	Turbid solution

Example 2: Preparation protocol for solution of Compound 1 in Captisol®

Standard dissolution methods, such as mixing dry Compound 1 and dry Captisol® into water or adding Compound 1 to a prepared solution of Captisol® and water did not result in complete dissolution at the desired concentrations. Several different concentrations of both Compound 1 and Captisol® were tested at various pH levels. However, the following method for producing a solution of Compound 1 in Captisol® resulted in complete dissolution at the desired concentrations.

Materials: Captisol®, Compound 1 and water

Method:

1. Weigh the appropriate amount of Captisol® to give a final concentration of 120 mg/ml.
2. Add 80% of the final amount of water and mix for 10 minutes with a magnetic stirrer.
3. Weigh Compound 1 to give a final concentration of 2.5 mg/ml, 2.0 mg/ml, 1.0 mg/ml, 0.5 mg/ml or 0.1 mg/ml.
4. Add the peptide to the Captisol® solution. Mix for 1 hour.
5. Raise the pH to obtain clear solution (in the 2.0 mg/ml formulation there might be a need to raise the pH slightly above 9). pH should be adjusted using HCl 1.0 N and NaOH 1.0 N. Mix for 10 minutes.
6. Correct the pH to the range of 7.5 to 8.5 if needed (using either HCl or NaOH 1.0 N).
7. Add water to final volume.

8. Filter the solution through a 0.2μ cellulose acetate filter.

9. Record final pH.

10. Dispense into aliquots and store at the proper temperature.

Example 3: Lyophilization of Compound 1 and Captisol® solution

The current lyophilization process differs from other lyophilization processes in that the percentage of solids in the formulation is high (12%) whereas lyophilized products normally contain between 5 and 10% solids.

Equipment

The freeze drier used was an Edwards lyophilizer Lyoflex 0.6. The equipment IQ/OQ was performed and checked for compliance by quality assurance prior to the process development.

Solutions of Compound 1 and Captisol® at concentrations of 0.5 mg/ml, 1.0mg/ml and 2.5mg/ml of Compound 1 were prepared. The fill-volume was adjusted 1 ml (1.05 gr).

Main process steps:

1. Freezing
2. Holding (at low temperature)
3. Drying under vacuum in two stages:
   3.1. Primary drying - shelf warming to an upper hold temperature, controlling shelf temperature at the upper hold level.
   3.2. Secondary drying - Pressure reduction to a minimal value at the upper hold shelf temperature.

Batches 1-3

Freezing - Freezing was from room temperature to -40°C within 2 hours. Shelves were held at -40°C for 3 hours.

Drying - Drying was performed at 110 μbar pressure. Shelf temperature was increased to 20°C over 13 hours and held at that temperature for additional 13 hours.

Total process time was 31 hours.

Results:

Water content results were:

Batch no. 1: 3.8%

Batch no. 2: 4.0% and

Batch no. 3: 4.9%

Batches 4 and 5

Since the water content results of the processes leading to batches 1, 2 and 3 were higher then the desired value, it was decided to add a secondary drying step at the same temperature and at low pressure.

Drying - Drying was performed at 110μbar pressure. Shelf temperature was increased to 20°C over 13 hours and held at that temperature for additional 13 hours (Batch 4) or 8 hours (Batch 5). Pressure was decreased to 10μbar for additional 5 hours.

Total process time was 36 hours.

Results:

Water content results were

Batch 4: Placebo: 3.0%,

1 mg/ml: 3.9%.

Batch 5: Placebo: 4.1%

Conclusions

As shown, a satisfactory lyophilization process for Compound 1 with Captisol® was developed. Due to the high percentage of solids and hence the condensed cake, the developed process is longer then the currently available lyophilization cycles for peptides and it exhibits an additional secondary drying stage. Table 4 summarizes the developed process.

Table 4

Step	Compound 1 (Peptide) with Captisol®
Loading	5°C
Freezing	2 hours to -40°C
Hold at low temp.	3 hours to -40°C
Primary Drying:	
Warm to 20°C	13 hours pressure 110μbar
Hold at 20°C	13 hours pressure 110μbar
Secondary drying:	
Hold at 20°C	5 hours pressure 10μbar
Storage at	-20°C
Process time	36 hours

Example 4.

Examination of th in-vivo biological activity of the lyophilized compound solution (DP, 1 mg/vial, 12% captisol®)

The biological activity was monitored by inhibition of IL-2 secretion from Compound 1 reference standard (RS) specific T-cells following subcutaneous (s.c.) treatment with the lyophilized compound solution, i.e. the drug product (DP), at two concentrations. The results of the treatment are compared to those of treating mice with Compound 1 (RS) in phosphate buffered saline (PBS). The results are shown in the tables below and in Figure 2.

Experimental design:

1. Immunization — Day 0
(Compound 1 RS emulsified with CFA, at all four footpads)

2. Treatment
(s.c. at the back of the neck, in 200 μl solution) — Day 0

3. In-vitro activation with: — Day 10
   a. Compound 1 RS at concentrations of 0; 0.5; 1; 2.5; 5; 10; 25; 50 and 100 μg/ml
   b. a peptide with the reverse order of amino acids of Compound 1 (negative control).
   c. Con A (positive control).

4. Incubation of culture for 20 hrs at 37°C in a humidified 5% CO_2 incubator.

5. IL-2 measurement by ELISA.

Table of experimental Groups:

Group	Immunization with	Treatment
A	50 μg Compound 1 RS	50μg Compd. 1 RS in PBS
B		200μg Compd. 1 RS in PBS
C		50μg DP(batch 2)
D		200μg DP (batch 2)
F		Placebo (12 % captisol®)

IL-2 Secretion from Compound 1 (DP) Treated Mice Following in-vitro Activation with Compound 1 RS (pg/ml)

			Treated with:							
	Group	**F**	**A**		**B**		**C**		**D**	
Activator	Concentration of activator (μg/ml)	12% captisol® Ampulized	Compd. 1 RS 50 μg/mouse	% inhib.	Compd. 1 RS 200 μg/mouse	% inhib.	DP 50 μg/mouse	% inhib.	DP 200 μg/mouse	% inhib.
Con A	2.5	5,825	6,215		5,403		3,537		4,069	
Compd. 1 RS	0	BQL	BQL		BQL		BQL		BQL	
Compd. 1 RS	0.5	11	9		10		8		BQL	
Compd. 1 RS	1	10	BQL		BQL		BQL		BQL	
Compd. 1 RS	2.5	15	8	51	BQL	NA	6	61	6	62
Compd. 1 RS	5	20	9	55	8	60	10	48	7	63
Compd. 1 RS	10	25	16	38	11	58	13	48	8	67
Compd. 1 RS	25	29	15	48	11	63	16	45	13	56
Compd. 1 RS	50	40	21	47	15	62	20	50	12	69
Compd. 1 RS	100	42	25	41	18	58	24	43	15	64
	Average inhibition (%) (at the range of 5-100 μg/ml)			**45.6**		**60.4**		**46.8**		**63.7**

BQL = Below Quantitation Limit

NA = Not Applicable

Rows 1-4 were not included in the curve

Example 5: Evaluation of Optimal Dose for Treatment

The following abbreviations are used in the following description:

CFA	Complete Freund's adjuvant
Con A	Concanavalin A
DP	Drug Product
DS	Drug Substance
EM-1	Enriched DCCM-1 Medium
EM-3	Enriched RPMI-1640 + fetal calf serum medium
FCS	Fetal Calf Serum
IFN-γ	Interferon-gamma
LN	Lymph Node
PBS	Phosphate Buffered Saline
RS	Reference Standard
s.c.	Subcutaneous
TB	Trypan Blue
TGF-β	Transforming Growth Factor-beta
WFI	Water for Injection

Introduction

A group of 20 mice were immunized with 50 μg/mouse of Compound 1 RS. The immunized mice were allocated to five treatment groups as follows: placebo, 25, 50, 100 and 200μg/mouse of Compound 1 DP (subcutaneous administration). Ten days post immunization and treatment, LN was extracted and single cell suspension was prepared. The *in-vitro* secretion of IFN-γ and TGF-β by the cultured cells in response to activation with several concentrations of Compound 1 RS was then measured.

Experimental design

1. Immunization -Day 0
2. Treatment with Compound 1 DP -Day 0

3. In-vitro activation of LN cells from treated mice -Day 10
4. Collection of culture media (for IFN-γ determination) -Day 12
5. Collection of culture media (for TGF-β determination) -Day 13
6. ELISA for IFN-γ
7. ELISA for TGF-β

Table 7: Experimental Groups

Exp. Group	Treatment		In-vitro activation	
	Article	**Mice/group**	**Cells/well**	**Compound 1 RS concentration**
A1	Control 12% Captisol®	4	2.5×10^6	Compound 1 RS 0-100 μg/ml
			5×10^6	
A2	25 μg/mouse	4	2.5×10^6	
			5×10^6	
A3	50 μg/mouse	4	2.5×10^6	
			5×10^6	
A4	100 μg/mouse	4	2.5×10^6	
			5×10^6	
A5	200 μg/mouse	4	2.5×10^6	
			5×10^6	

Materials and Reagents

Animals

Mice: 20 female BALB/c mice, supplied by Harlan animals breeding center, Rehovot.

Age at immunization (week+days): 10

Average weight of mice included in the experiment: 19.01 gr.

Materials

General reagents

70% ethanol was prepared from 96% ethanol by diluting with purified H_2O.

Preparation of Compound 1 solutions for immunization

CFA-Compound 1 RS emulsion (500 μg/ml, 50 μg/mouse) was prepared as follows:

1. 1.874 mg of Compound 1 was dissolved in 1.87 ml of WFI to yield a solution of 1 mg/ml.
2. The solution was tested with a pH indicator strip and found to have a pH of 5.
3. 1.5 ml of the solution were emulsified with 1.5 ml CFA resulting in a final concentration of 500 μg/ml.

Preparation of solutions for Treatment

Treatment was by a s.c. injection of 200 μl solution.

Preparation of 12% captisol® solution

1.2 gr of captisol® were dissolved in 10 ml of WFI to yield a solution of 12% captisol®.

Experimental procedure

Mice weighing

Mice were weighed before immunization. Average mice weight: 19.01 ± 0.97 gr

Immunization

The immunization was performed by injecting 100 microliters of the emulsion (50 microliters into each hind footpad).

Treatment

Following the immunization step the mice were treated by s.c. injection of 200 μl from the designated Compound 1 DP or 12% captisol® treatment solutions, at the back of their neck.

In-vitro cultur

Mice were sacrificed by cervical dislocation. LN were extracted from the hind legs and were transferred to a sterile petri dish containing about 5 mL RPMI. The cells were extracted by gentle squeezing of the tissue against a 200 micrometer mesh stainless steel net. The cells were collected and centrifuged at 300 G for 10 minutes at RT.

Single cell suspensions were prepared from pooled LN of each experimental group.

2.5 and 5.0 million cells/ml/well suspensions were cultured with Compound 1 RS (0-100 μg/ml) in EM-1.

Secretion of IFN-γ and TGF-β, as indication of cellular response, were determined by ELISA of culture media (48hrs for IFN-γ and 72hrs for TGF-β).

Table 8: The *in-vitro* experimental groups

Experimental Group	Treatment	In-vitro activation	
	Article	Cells/well	Activation substance concentration
A1-2.5	Control 12% Captisol®	2.5×10^6	Compound 1 RS 0; 3.125; 6.25; 12.5; 50 and 100 μg/ml Con A 2.5 μg/ml
A1-5		5×10^6	
A2-2.5	DP 25 μg/mouse	2.5×10^6	
A2-5		5×10^6	
A3-2.5	DP 50 μg/mouse	2.5×10^6	
A3-5		5×10^6	
A4-2.5	DP 100 μg/mouse	2.5×10^6	
A4-5		5×10^6	
A5-2.5	DP 200 μg/mouse	2.5×10^6	
A5-5		5×10^6	

Preparation of cell suspensions

Table 9: Results of cell counting and preparation of cell suspensions (10 x 10^6/ml)

Grp	Vol. (ml)	Dilutn. factor	Viable cells	Dead cells	%Viable cells	%Dead cells	Average viable cells	Total viable cells (x 10^6)	EM-1 to add (ml) for suspension of 10 x 10^6 cells/ml
A1	10	16	115	--	100	--	112	179.2	17.9
			109	--	100	--			
A2	10	16	50	4	92.6	7.4	47.5	76	7.6
			45	2	95.7	4.3			
A3	10	16	80	4	95.2	4.8	80.5	128.8	12.8
			81	5	94.2	5.8			
A4	10	16	87	--	100	--	89	142	14.2
			91	--	100	--			
A5	10	16	120	2	98.4	1.6	112.5	180	18
			105	2	98.1	1.9			

Preparation of cell suspensions (5 x 10^6/ml)

The 10 x 10^6 cells/ml suspensions were diluted 1:2 by adding 5 ml EM-1 to 5 ml cells suspension.

Incubation of LN cells cultures in 48 wells plates

3 tissue culture plates were prepared. The following was added to each plate.

Background control (cells incubated with culture media)

0.5 ml of cells suspension

0.5 ml of culture media (EM-1)

System positive control (cells stimulated with Con A)

0.5 ml of cells suspension

0.5 ml of Con A 5 μg/ml in EM-1 (final conc. 2.5 μg/well)

Cells incubated with Compound 1 activation solutions (samples)

0.5 ml of cells suspension

0.5 ml of Compound 1 RS 6.25 - 200 μg/ml (final conc. 3.125 - 100 μg/ml/well)

Incubation of LN cells cultures in 96 wells plates

After the 48-wells plates were prepared, 96-wells plates were prepared by applying 100 μl from the cell suspension and 100 μl from the activation solutions.

The culture plates were incubated at 37° C in a humidified 5% CO_2 incubator, for either 48 or 72 hrs.

Supernatants Collection

The cultured plates were centrifuged at 300 g for 10 minutes at RT. Supernatants (850 μl from each well) were transferred either to mirror plates or to tubes. The supernatant was then divided into working aliquots (two aliquots of 200 and one aliquot of 450 μl), in order to avoid repeated freeze/thawing of the samples. Each tube was labeled with the following details:

1. Experimental code and time post incubation.
2. Group and sample number
3. Activator and concentration.
4. Date of sup collection

The supernatants were stored at -20°C until used for ELISA.

Results

Table 10: Summary of Groups

Experimental Groups:				
Immunization		Treatment		In-vitro activation
Exp.Groups	Immunization dose	Sub group	Article	
A	50μg/mouse	A1	12% Captisol® Placebo control	Compound 1 RS 3.125-100μg/ml
		A2	Compound 1 25μg/M	
		A3	Compound 1 50μg/M	
		A4	Compound 1 100μg/M	
		A5	Compound 1 200μg/M	

Table 11-A: Final cytokine concentrations

Final cytokine (pg/ml) (2.5 million cells/well)

Compound 1 concentration	Placebo	50µg/M	100µg/M	200µg/M
3.125μg/ml	321.3	54.1	64.5	103.9
6.25μg/ml	238.6	81.8	116.1	126.1
12.5μg/ml	397.1	123.1	180.9	129.0
25μ/ml	655.5	215.1	262.8	240.3
50μg/ml	573.9	292.5	518.3	378.1
100μg/ml	926.0	531.8	582.7	524.1
Con A	322.6	356.2	337.4	BQL

Table 11-B: Final cytokin concentrations

Final cytokine (pg/ml) (5 million cells/well)

Compound 1 concentration	Plac b	25µg/M	50µg/M	100µg/M	200µg/M
3.125µg/ml	522.3	BQL	76.2	90.8	204.4
6.25µg/ml	634.8	BQL	109.2	157.8	244.1
12.5µg/ml	962.8	41.9	179.5	257.1	466.1
25µ/ml	967.4	70.0	277.9	421.7	660.5
50µg/ml	1338.8	104.2	373.4	739.7	922.5
100µg/ml	2010.2	185.2	547.0	995.5	1006.2
Con A	6839.8	2995.3	4837.0	10126.8	7722.8

The results are also presented in Figures 3-4.

Observations

IFN-γ secretion

1. In the placebo group, a linear dose response upon Compound 1 activation in-vitro was shown. This graph resembles the graph obtained for the Ex-vivo model with the same immunization dose (50µg/mouse) and culturing medium (EM-1).
2. There was a dose response upon Compound 1 activation in vitro within all the tested groups.
3. Significant inhibition of IFN-γ secretion was seen with all the doses used for treatment (an average of 95% inhibition with treated dose of 25µg/mouse). A reverse correlation between the dose served for treatment and % inhibition can be found, mainly when $5x10^6$ cells/well were used. When $2.5x10^6$ cells/well were used, treatment of animals with 50µg/mouse gave better inhibition than 100 or 200µg. The point of 25µg is missing (lack of cells).
4. A better inhibition was seen when $5x10^6$ cells/well were used instead of $2.5x10^6$ cells/well.
5. In the linear range of the graphs, SD of % inhibition was low.
6. A technical problem with Con A is apparent when $2.5x10^6$ cells/well were used.

TGF-β secretion

1. In the placebo group, no dose response upon in vitro activation with compound 1 was seen. TGF-β secreted level was below the detection limit of the ELISA in all other treatment groups.

Example 6: Optimizations of freeze drying cycle with Compound 1 and Captisol® for injection (0, 0.5, 1.0 and 2.5 mg/vial)

Purpose

The purpose of this study was to optimize the freeze drying cycle for Compound 1 with captisol® for injection to improve the shape of the lyophilization cake and avoid collapse and cracking. Thus it was decided to improve and optimize the lyophilization cycle. This cycle is transferred to the production lyophilizers for the manufacturing of the phase I batches.

Process optimization

Batches of peptide at concentrations of 0.5 mg/ml 1.0mg/ml, 2.5mg/ml and Placebo were prepared and several freeze drying cycles were performed. The freeze drier used was an Edwards lyophilizer Lyoflex 0.6.

Solubility, water content and cake appearance were tested.
According to the obtained results a new lyophilization cycle for Compound 1 was selected. Due to the high percentage of solids (12%) and hence the condensed cake, the new process is longer than the lyophilization cycle in Example 3 and exhibits an additional primary drying stage. Table 12 summarizes the differences between the processes.

Table 12

Step	Lyoph. cycle for Compound 1 and Captisol® of Example 3	**New Lyoph. cycle for Compound 1 and Captisol®**
Loading	5°C	**5°C**
Freezing	2 hours to -40°C	**6 hours to -45°C**
Hold at low temp.	3 hours to -40°C	**3 hours to -45°C**
Primary Drying: Stage I **Stage II** Hold at 20°C **(25°C)**	to 20°C 13 hours pressure 110μbar - 13 hours pressure 110μbar	**to -20°C** **19 hours pressure 150μbar** **to 25°C** **13 hours pressure 150μbar** **8 hours pressure 150μbar**
Secondary drying: Hold at 20°C	 5 hours pressure 10μbar	 -
Storage at	5°C	**5°C**
Process time	36 hours	**49 hours**

Example 7: Effect of Compound 1 (administered in Captisol®) on lupus symptoms in the SLE-prone (NZBxNZW)F1 female mouse

Patients participating in clinical trials are to be treated with Compound 1 using Captisol® (sulfobutyl ether beta-cyclodextrin sodium) as the excipient. For this reason, it was important to determine whether treatment of (NZBxNZW)F1 mice with the formulation of Compound 1 given in Captisol® would have the same beneficial effects on lupus symptoms as observed when this strain of mice was treated with Compound 1 in PBS.

To this end, (NZBxNZW)F1 female mice (about 8 months old) were divided into 3 groups that were treated subcutaneously once a week for 10 weeks either with Captisol® alone (n=8) or with 25 or 50 μg/mouse Compound 1 in Captisol® (n=9 and 10, respectively). These doses were selected since prior studies indicated that doses in this range were more effective in ameliorating SLE symptoms than the higher doses tested (100 and 200 μg/mouse). The

same batch of drug substance was used in this study and in the first Phase I clinical trial with Compound 1.

The mice were followed for anti-dsDNA antibodies and for proteinuria. When the mice were sacrificed, the intensity of ICD was determined in kidneys.

As can be seen in Figure 5, no significant differences between groups could be observed in the levels of dsDNA-specific antibodies after 10 treatment injections.

Table 13 also shows that the beneficial effect of treatment with Compound 1 could be observed starting from the 5th injection and it was sustained up to the 10th injection. The mean levels of proteinuria in the Captisol® control group were consistently higher than in the Compound 1 -treated groups. Table 13 also shows that a reduction in the intensity of ICD was observed in kidneys of both Compound 1 dose groups. There was an overall trend showing that the lower dose (25 μg/mouse) was more effective than the higher dose (50 μg/mouse) in reducing the clinical symptoms of SLE in these mice.

Table 13. Clinical Symptoms of SLE in (NZBxNZW)F1 Mice Treated with 25 or 50 μg/mouse Compound 1 (in Captisol®)

Study Group	Mean Proteinuria ± SEM (g/L) Number of Weeks Following Treatment Initiation				ICD[a] (Mean ± SEM)
	5	7	8	10	
Captisol®	1.81 ± 1.22 (n=8)	5.74 ± 3.13 (n=8)	4.5 ± 2.92 (n=7)[b]	4.46 ± 2.93 (n=7)[b]	2.29 ± 0.28 (n=7)
Compound 1 (50 μg/mouse)	0.75 ± 0.3 (n=10)	0.81 ± 0.3 (n=10)	1.09 ± 0.4 (n=10)	1.29 ± 0.3 (n=10)	1.90 ± 0.23 (n=10)
Compound 1 (25 μg/mouse)	0.16 ± 0.05 (n=9)	1.26 ± 1.09 (n=9)	0.5 ± 0.31 (n=9)	0.56 ± 0.3 (n=9)	1.22[c] ± 0.32 (n=9)

[a] ICD=Immune Complex Deposits. ICD intensity scale: 0=none; 1=moderate; 2=severe; 3=severe/extremely intense.

[b] The death of one animal with a high level of proteinuria resulted in a lower group mean.

[c] $p<0.05$ (compared to Captisol®-treated control mice; Mann-Whitney).

Figure 6 shows representative sections of one kidney from each treatment group. The top row sections are from a Captisol®-treated mouse, the mid-row sections are from a mouse treated with 50 μg/mouse Compound 1 and the bottom row sections are from a mouse treated with 25 μg/mouse Compound 1. It can be seen that the intensity of immune complex deposits observed in kidney sections of mice treated with Compound 1 (dissolved in Captisol®) at either dose level was much lower than that observed in the control group.

Example 8: Phase I Clinical Study

A Phase I, Multicenter, Randomised, Double-Blind, Placebo Controlled, Single Dose, Four-Arm Study to Assess the Tolerability and Safety of Compound 1 in Captisol® Subcutaneous Injection in SLE Subjects.

This was the first clinical study with Compound 1 in captisol® in humans, conducted in France. Its main objective was to evaluate tolerability and safety of Compound 1 in captisol®, administered as a single sc injection to SLE subjects. Its

secondary objective was to evaluate immunological responses following a single sc dose of Compound 1 in captisol® in these subjects.

Thirty-six (36) subjects participated in the study. To be eligible for inclusion in the study, SLE patients must have fulfilled at least four criteria used for the diagnosis of lupus by the American College of Rheumatology. Patients must also have had stable, mild/moderate disease and score less than or equal to 10 on the SLE Disease Activity Index, SLEDAI.

Each patient received a single sc injection of reconstituted Compound 1 for injection or its matching placebo (Captisol®) according to the following group assignment:

- ***Group A***: Placebo (Captisol®)
- ***Group B***: 0.5 mg Compound 1 in Captisol®
- ***Group C***: 1 mg Compound 1 in Captisol®
- ***Group D***: 2.5 mg Compound 1 in Captisol®

A standard battery of safety tests, including blood and urine collection for laboratory tests, was performed at screening, during the day of dosing, at 24 hours post-dose and at 2, 4 and 8 weeks following dosing. Prior to dosing, and on scheduled follow-up visits, blood samples were withdrawn for SLE-related immunological tests, anti-Compound 1 antibodies and PBL proliferation assay. The following immunology tests were performed:

- Coomb's (direct and indirect)
- C3, C4 and CH50
- Total IgG, IgM and IgA
- ANA, anti-dsDNA (Farr assay), anti-ssDNA
- Anti-ENA (including anti-La, anti-Ro, anti-RNP, anti-Sm)
- Anti-cardiolipin antibodies
- VDRL

- FTA antibodies
- Rheumatoid factor

The safety and tolerability of Compound 1 in captisol® in the subject population was evaluated on the basis of the following criteria:

- Occurrence of AEs, including SLE flare
- Vital signs
- 12-lead ECG
- Changes in physical examination
- Routine clinical laboratory tests
- SLEDAI score
- Immunological test results

Phase Ia clinical study details

Study Principal Investigators and Respective Study Sites: Six (6) study centers in France: Prof. Jean Charles Piette (Hopital La Pitie Salpetriere, Paris), Prof Oliver Meyer (Hopital Bichat, Paris), Prof. Jean Revuz (Hopital Henri Mondor, Creteil), Prof. Loic Guillevin (Hopital Avicenne, Bobigny), Prof. Eric Hachulla (Hopital Claude Huriez, Lille Cedex), Prof. Xavier Mariette (Hopital Bicetre, Kremlin Bicetre).

Compound 1 (in captisol®), Placebo, Water for Injection-Ampoules, Dose and Mode of Administration:
Vials of Compound 1 in Captisol® (120mg/vial) were injected subcutaneously as a single dose per subject in the following dosages:
0.5 mg Compound 1/vial in Captisol®, 1mg Compound 1/vial in Captisol® and 2.5 mg Compound 1/vial in Captisol®.
Placebo for Compound 1: 120 mg Captisol®/vial (identical in appearance to vials of Compound 1 in Captisol®).

Methodology

This was a multi-center, randomized; double blind, placebo-controlled, four-arm study, using a single subcutaneous injection of Compound 1 or placebo. SLE patients were screened up to 21 days prior to baseline procedures. Each eligible subject was randomized to one of the 4 treatment groups: subcutaneous injection of either 0.5, 1 or 2.5 mg Compound 1 or its matching placebo. All subjects were admitted to the clinic on pre-dosing day. Each subject received a single dose of one of the above listed treatments. Subjects were discharged from the clinic 24 hours after dosing. Subjects were further monitored at weeks 2, 4 and 8 following dosing. Blood samples (serum and whole blood) for safety laboratory tests were withdrawn at Screening, Dosing Day (pre-dose), Day 2 (post dose), at Weeks 2, 4 and 8 (Termination visit). Blood samples for immunological tests were withdrawn at: Screening, Dosing Day (pre-dose) and at Weeks 4 and 8. Peripheral blood lymphocytes (PBL) proliferation was evaluated at Dosing Day (pre-dose) and at Weeks 2, 4 and 8.

Number of Subjects (total and for each treatment):
Thirty six (36) subjects were randomized into this study as follows; 9 subjects into the 0.5 mg treatment group, 9 subjects into 1 mg treatment group, 10 subjects into the 2.5 mg treatment group, and 8 subjects into the placebo treatment group.

Diagnosis and Main Criteria for Inclusion:
Eligible subjects for this study were SLE patients who fulfilled at least four diagnostic criteria of the American College of Rheumatology (ACR). Their disease condition had to be stable, mild to moderate with a score equal to or less than 10 on the SLE disease activity index, year 2000 updated (SLEDAI 2K).

Excluded from participation were SLE patients who reported unstable or severe asthma, stroke, acute myocardial infarction, unstable angina, cerebral hemorrhage and pulmonary embolism during the six months prior to study screening. SLE patients

who had any clinically significant or unstable medical or surgical conditions, diabetes mellitus, liver disease (cirrhosis, active hepatitis, portal hypertension, and/or ascites), clinically significant hypertension, a medical history of any malignancy, dialysis, or chronic obstructive pulmonary disease (COPD) were also excluded from study participation.

Also excluded from study participation were SLE patients who underwent plasmapheresis or were treated during the three months prior to screening with one of the drugs listed below: prednisone 30mg/day or greater (or an equivalent dose of another corticosteroid), intravenous corticosteroids, intravenous immunoglobulin G (IgG), oral anticoagulants and any cytotoxic agents (e.g. azathioprine, chlorambucil, cyclophosphamide, mycophenolate mofetil, methothrexate, tacrolimus.

In addition, SLE patients initiating treatment with corticosteroids (more than ± 10 mg/day prednisone, or an equivalent dose of another corticosteroid) and/or anti-malarials, during the last 3 months prior to screening were excluded from the study.

While an effort was made to retain baseline SLE medical treatments throughout the course of the study, investigators could nevertheless change participant medical treatment at any time during the study to maintain and optimize patient welfare.

Criteria for Evaluation

Safety:

The following safety parameters were assessed at Screening, during the hospitalization and at follow-up visits including Termination visit: vital signs (systolic blood pressure, diastolic blood pressure, pulse, oxygen saturation, temperature and weight), 12-lead ECG, change in physical examination and

clinical routine laboratory safety tests. Adverse events were recorded at the Dosing Day and at each visit thereafter.

Immunology:

SLE-related immunological tests were performed at Screening, during the hospitalization and at follow-up visits including Termination visit.

Drug-related immunological responses were followed by using the PBL proliferation assay and anti-Compound 1 antibodies assay at the Dosing Day and at follow-up visits including Termination visit.

Disease Activity:

Disease activity assessment using the SLE disease activity index score, year 2000 updated (SLEDAI 2K) was assessed at Screening, during the hospitalization and at follow-up visits including Termination visit.

Statistical Methods:

SAS® version 9.0 software was used to analyze and present data collected during this study. No power calculation was performed and no formal hypothesis testing was conducted for this Phase Ia study.

Adverse Experiences

The incidence and the frequency of adverse experiences was presented by System Organ Class and preferred terminology according to MedDRA dictionary version 5.0. The data is tabulated by treatment group.

Clinical Laboratory Data

Descriptive statistics of laboratory values including number of observations, mean, standard deviation, minimum and maximum were determined for Screening, Day 1 (pre dose), Day 2, Week 2, 4 and

8 are presented by treatment group. Changes from baseline to each time point/visit are also presented for each visit by treatment assignment. Percent of abnormal results (low and high, where applicable) are presented on a parameter basis, by treatment group and visit/time point. Shift analyses from baseline to 24-hours post dose and from baseline to termination visit were performed.

Vital Signs

Descriptive statistics for vital signs including number of observations, mean, standard deviation, median, minimum and maximum values were determined for Screening, Day 1 (pre and post dose, and at each time point) Day 2, Weeks 2, 4 and 8 are tabulated by the assigned treatment. Changes from baseline to each time point/visit is presented in by visit and treatment assignment.

Weight

Descriptive Statistics of Weight (kg) at baseline, termination and change from baseline is presented by treatment group.

ECG

Descriptive statistics of ECG parameters at baseline, termination and changes from baseline are presented. Shift analysis is presented as tables of shift from baseline to termination between normal/abnormal or present/absent ECG parameters. Potentially clinically significant (PCS) QTc (Bazett) measurements were identified according to the predefined criteria. Tables of shift analysis between PCS and non-PCS Absolute QTc (Bazett) and incidence table of PCS change in QTc (Bazett) from baseline to any visit are presented.

Physical Examination

Physical examination results are analyzed by incidence of subjects with abnormal or normal findings for each body system

at Baseline and Termination visit. Shift analysis between normal to abnormal and vice versa was also applied. When no change from baseline occurred, it was defined as "other".

Compound 1 related immunological tests

For immunological parameters, descriptive statistics, including number of observations, mean, standard deviation, median, minimum and maximum values were calculated and are presented by treatment group and visit. Change from baseline to each follow-up visit is also presented by treatment group. Where applicable, number and percent of subjects with negative/positive results is presented by treatment group and visit.

SLEDAI 2K

Descriptive statistics, including mean, standard deviation, median, minimum and maximum values of SLEDAI 2K are presented.

Results of Phase Ia clinical study:

<u>Subject Disposition and SLE Characteristics</u>

Thirty six (36) study subjects entered and completed this study per protocol. The majority of subjects (34) in all treatment groups were female (94.4%) and Caucasian (30, 83.3%). The mean age for all treatment groups was 35.6 years (range of means from 32 to 39 years). Most of the subjects (91.7%) had between 4 to 6 American College of Rheumatology (ACR) diagnostic criteria and a mean group SLEDAI 2K score ranged from 2.1 to 4.1.

<u>Safety Results</u>

There was no prominent difference between study drug treatment groups and the placebo group in the incidence of AEs. The most common AEs in all groups were headache, classified as mild or moderate in nature and injection site reaction classified as mild in nature. Dose response was not seen. No serious adverse event (SAE) or AE classified as severe occurred during the study.

No clinically significant effect attributable to study drug was seen for hematology, biochemistry or urinalysis values.

No clinically significant effect attributable to the study drug was seen for vital signs parameters (systolic blood pressure, diastolic blood pressure, pulse, oxygen saturation).

No clinically significant effect attributable to the study drug was seen for temperature and weight.

No differences of clinical significance were seen between Compound 1 -treated groups and placebo for categorical ECG measurements and digitized ECG parameters. No PCS QTc absolute value and no QTc change from baseline > 60 msec was recorded. A similar number of subjects in Compound 1 -treated and placebo groups had QTcB change from baseline between 30 and 60 msec.

No clinically significant effects of Compound 1 on physical exam were noted.

Immunology Results

Evaluation of serum samples from all subjects indicated that a single subcutaneous administration of Compound 1 at the dose levels of 0.5, 1 and 2.5 mg/patient did not induce the development of anti-Compound 1 specific antibodies. Seven subjects had a response to Compound 1 above the cut-off. These elevated levels of antibodies were already present prior to dosing. No increase in the levels of antibodies was observed in the follow up period (two months) of the study. The sera of these subjects were analyzed for the isotype of the reactive antibodies. The response in two of the subjects was associated with the IgM isotype and with the IgG isotype in two others. None of the seven had specific IgE antibodies.

The peripheral blood lymphocytes (PBL) assay showed that 50 % of the subjects (18) were classified as responders (SI>2) with similar distribution in all treatment groups. The T cell response was relatively low and no association between Compound 1 treatment dose or concentration used in the assay and responder/ non-responder status could be detected, taking into consideration that only a single SC dose of the study drug was administered. Also, no indication of increased incidence of responder status over time was observed. The tetanus toxoid (TTX) assay that serves as a safety control shows that the response to TTX was preserved throughout the study period in all treatment groups indicating that Compound 1 in captisol® did not change the immunological response to TTX recall antigen.

The immunological findings are the result of the administration of only a single dose of the study drug Compound 1.

Disease Activity Results

No clinically significant effects of Compound 1 on the SLEDAI score (change of ≥ 3, ≤ 12 points) were noted during the study except for one subject in the 0.5 mg treatment group for whom a change in the SLEDAI score of 2 to 10 points was recorded between baseline and week 4 on the basis of an urinalysis showing pyuria. This urinalysis finding was not confirmed by the investigator as a lupus flare per protocol definition and was resolved with no treatment change.

Conclusions

This Phase Ia study showed that a single subcutaneous injected dose of Compound 1 of 0.5, 1 or 2.5 mg in 120 mg Captisol ® was safe and well tolerated and allows continuation to a phase Ib multiple dose study.

Example 9: Phase Ib Clinical Study

A Phase I, Multic nter, Bi-National, Randomized, Double-Blind, Four-Arm, Placebo Controlled, Multiple Dose Study to Assess the Tolerability and Safety of Compound 1 in Captisol® Subcutaneous Injections in SLE Subjects

This study is being performed in order to evaluate the safety and tolerability of repeated Compound 1 sc administration to SLE subjects. The study's secondary objective is to evaluate immunological responses following repeated sc administration of Compound 1 in Captisol® in SLE subjects.

Compound 1 is given in doses of 0.5, 1.0 or 2.5 mg in Captisol®. The investigational product is administered every other day (excluding weekends) for a total of 12 sc injections, i.e. 3 doses a week for 4 weeks. Subjects are monitored on planned visits scheduled at 2, 4, 8 and 12 weeks after start of dosing. Safety and tolerability are evaluated using tests similar to those described in the Phase Ia Clinical Study above.

Results

This Phase Ib study shows that multiple subcutaneous injected doses of Compound 1 of 0.5, 1 or 2.5 mg in 120 mg Captisol ® are safe and well tolerated.